CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

CYCLOATE

Chemical Code # 516, DPN # 212
SB 950 # 336

July 13,1987
Revised 3/11, 10/3, 11/9/88, 2/21, 7/24/90,
3/21/91, 5/3/91, 4/10/92, 10/25/93, 11/2/93, 12/27/94, 10/9/98

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect (not oncogenicity)
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, possible adverse effect
Chromosome change:	No data gap, possible adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	No data gap, possible adverse effect

Toxicology one-liners are attached.
Summary reconciled with library printout dated 12/08/94. All record numbers through 133350 (Document No. 212-078) were examined. Additional volumes include -87 through 92, record number 154954.
In the one-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T981009
Previous Summaries by Luthra, 3/11/88; Davis, 10/3/88 & 11/9/88; Stanton Morris, 2/21/90; G. Chernoff, 7/24/90; J. Gee, 3/21/91 & 5/3/91; T. Moore, 4/10/92; and Kellner, 10/25/93 and 11/2/93. Aldous and Kellner, 12/27/94. J. Gee, 10/9/98.

These pages contain summaries only. Individual worksheets may contain additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

009 024960 and 011 037062, "24-Month Chronic Feeding Study in Rats RO-NEET Technical Final Report ", (Hazleton Laboratories, 5/25/79). Cycloate technical, 95.3%, was fed in the diet to Sprague-Dawley rats, 60/sex/group at 0, 8, 24 or 72 mg/kg/day for 106 weeks. Ten/sex/group were sacrificed at 53 weeks; NOEL < 8 mg/kg/day (dose-related neuromyopathy of sciatic nerve and of muscle; posterior paralysis observed at mid and high doses). UNACCEPTABLE (no individual clinical observations, dose selections with all too high). (Gee 1/24/86).

030 059841, Addendum to record # 037062. Individual animal data for body weights and food intake were reviewed for the SB950 Response to CDFA's question following the evaluation of the 24-Month Chronic Feeding Study in Rats, RO-NEET (record # 037062). The response was dated 17th June 1987 (document # 212-031). There is no change in the status, the collective data in record #'s 24960, 37062, 24959 and 37063 to 37068 fulfilled the requirements. Luthra, 3/11/88

009 024959 and 012 to 017 037063 to 037068, "Two Year Oral Toxicity Study With RO-NEET Technical in Rats: Final Report ", (Stauffer Environmental Health Center, 3-7-84). Cycloate Technical, 98%, lot CGB-2201, EHC 0009-06, was fed to groups of 70 Sprague-Dawley rats/sex at 0, 2, 10, 60 or 300 ppm in the diet for 2 years; NOEL = 10 ppm (neuromyopathy). Study was designed to establish this NOEL following finding reported in Record Nos. 24959 and 37062 (incomplete combined study). UNACCEPTABLE, study does not address full pathology. (Gee 1/24/86).

SUMMARY: Although each study was found unacceptable due to flaws in the design or in reporting of data, CDFA believes that collectively they provide sufficient data to determine that cycloate results in a potential adverse effect with a nominal NOEL of 10 ppm for neuromyopathy. (Luthra, 3/11/88).

CHRONIC TOXICITY, DOG

**** 034 066035**, "One Year Chronic Oral Toxicity Study With RO-NEET in Beagle Dogs", (Stauffer Chemical Co., Farmington, CT., Report # T-12635, 10/21/87). Cycloate, 97.6% purity, was administered orally by intubation in corn oil (dose volume 0.5 ml/kg) or as neat material to 5-6 month old beagle dogs, 4/sex/group at 0, 0.5, 50, 200 & 850 mg/kg for 1 year and 3/sex/group at 0 and 1200 mg/kg for 3 months (interim sacrifice, necropsy limited to neuro-pathology). **Possible adverse effects:** Neuromyopathology, dilatation of cerebral ventricles and Wallerian degeneration elicited at 200 mg/kg & higher doses. Toxicity of liver-hypertrophy, fibrosis, inflammation; kidney-papillopathy, pyelopathy, pyelonephropathy; adrenals-cortical hyperplasia/hypertrophy; testis-degeneration of spermatogenic cells; focal myocardial degeneration/atrophy; and inhibition of growth was evident at 50 mg/kg and above. NOEL = 0.5 mg/kg (hepatic, renal & adrenal effects). ACCEPTABLE. (Luthra, 3/7/88).

021 049843, This document contains comments on CDFA evaluation of and individual body weights and food consumption for doc. # 212-034, rec. # 066035.

ONCOGENICITY, MOUSE

008 024957 and 018 037077, "Lifetime Oral Study in Mice", (IRDC, 11/8/79). RO-NEET technical, 97.2%, lot no. CEK 2001, or 95.3%, lot no. NATX 26035, was administered in the diet to 60/sex/group at 0, 20, 60 or 180 mg/kg/day for 104 weeks, interim sacrifice of 10/sex/group at 52 weeks. Nominal NOEL $\geq$ 180 mg/kg, no toxic effect reported at any dose. UNACCEPTABLE, need dose justification and diet analyses for early part of study. (Gee 1/27/86).

**** 047 089249** "Cycloate: 18 Month Carcinogenicity Study in Mice." (M. D. Stonard, ICI Central Toxicology Laboratory, UK, Report CTL/P/3125, 2/10/91) Cycloate, 98.1%, was fed in the diet to C57BL/10JfCD-1/Alpk mice, 50/sex/group at 0 (diet), 300, 1000 or 3000 ppm for 18 months. The mice were housed 5/cage with a 12/12 hour light cycle. There were no findings for oncogenicity effects. Reduced weight gain and food consumption in both sexes at 3000 ppm, especially early in the study, increased liver/body weight in the high dose were noted. **Possible adverse effects:** Increased incidence of "inactive" ovaries at 3000 ppm and blood filled cysts at 1000 and 3000 ppm, There was no significant evidence of neuromyopathy. Negative for oncogenicity. ACCEPTABLE. Gee, 5/2/91.

REPRODUCTION, RAT

**** 019 037080**, "Three Generation Reproduction Study in Rats ", (Goldenthal, E.I., IRDC, Study T-6340, 1/11/79). RO-NEET technical, 3 lots with purities of 97.2%, 95.3% and 95.3%, were administered in the diet to groups of 15 male and 30 female Charles River CD rats at dose levels of 0 (diet control), 8, 24 or 72 mg/kg/day for 3 generations, 2 litters per generation. At 72 mg/kg/day, there was a decrease in parental and pup body weight and a decrease in pup survival. At 24 mg/kg/day, parental and pup body weights remained reduced. Parental NOEL = 8 mg/kg/day (reduced body weight); Reproductive NOEL = 8 mg/kg/day (decreased pup weight) and a **POSSIBLE ADVERSE HEALTH EFFECT** (decreased pup weight and survivability) is noted. The study was originally reviewed as unacceptable by Gee 8/26/85 & Parker 1/29/86, but upgraded to ACCEPTABLE with the submission of record 065862. (B. Davis, 9/29/88; NOEL's revised by G. Chernoff, 7/20/90).

008 024956, Partial copy of 037080.

033 065862, Thirteen volumes of supplemental material (diet preparation data, diet analyses, breeding records, litter records, histomorphologic observations, statistical analyses, individual body weights and food consumption data, and necropsy data; Discussion by J. L. Minor. Davis 9/29/88, Revised 11/3/88.

**** 044 095692** "A Two-Generation Reproduction Study in Rats with R-2063." (Minor, J. L. and J. C. Turnier, Ciba-Geigy, Environmental Health Center, 10/26/90). Cycloate technical (R-2063, lot EHC-0952-05, 98.1%) was fed in the diet to Crl:CD(SD)BRVAF/plus rats, 25/sex/group, at 0, 50, 400 or 1000 ppm [approximately equivalent to 2.5, 20 and 50 mg/kg/day], two generations, two litters per generation. F0 and F1 breeding adults were examined for microscopic effects on the reproductive organs, nervous system, liver and kidneys. Body weights of parental males and females were significantly reduced at 400 ppm and at 1000 ppm (both sexes and generations with selected pups for the production of the F2 generations starting with a significant weight decrement). Effects on pups/litters were decreased survival from days 0 - 4 at 1000 ppm, lower body weights in F1A litters at day 0 at 400 and 1000 ppm, smaller litters in all 4 determinations at 1000 ppm. Systemic effects seen in microscopic examination were

mineralization of the brain and biliary hyperplasia (both sexes, 1000 ppm), thoracic and sacral spinal cord degeneration (females, 400 and 1000 ppm). Parental and reproductive NOELs = 50 ppm (reduced body weight gains, sacral spinal cord degeneration, biliary hyperplasia) **POSSIBLE ADVERSE EFFECTS.** ACCEPTABLE. (Gee, 1/9/91)

TERATOLOGY, RAT

** 008 024958 and 019 037079, "A Teratology Study in Rats with RO-NEET", (WIL Research Laboratories, 1/24/85). RO-NEET, 96.8%, lot no. 4921-12-9, was administered by gavage to groups of 25 Charles River COBS rats at 0 (corn oil), 10, 75, 175 or 400 mg/kg/day on days 6-15 of gestation. Maternal NOEL = 75 mg/kg (clinical signs, body weight); Developmental NOEL > 400 mg/kg (none noted). No adverse effect; ACCEPTABLE**.** (Gee 8/23/85, Parker 2/4/86).

TERATOLOGY, MOUSE

008 024955 and 019 037078, "Ro-neet Safety Evaluation by Teratological Study in the Mouse", (Woodard Research Corp., 4-6-67). Cycloate, 97.7%, lot no. OD-174-11, was fed in the diet to groups of 20 mice at 0, 8 or 24 mg/kg on days 6 - 18 of gestation. Maternal and Developmental NOEL $\geq$ 24 mg/kg; no clinical signs of maternal or developmental toxicity. No adverse effect; UNACCEPTABLE. No justification of dose, no evidence of MTD, inadequate data on skeletal and visceral findings. (Gee, 88/23/85, 1/22/86).

TERATOLOGY, RABBIT

** 063 119669, "Cycloate: Developmental Toxicity Study in The Rabbit", (J.M. Horner, ICI Central Toxicology Laboratory, Report No. CTL/P/3810, Study no. RB0589, 11/13/92). Cycloate, purity 95% w/w, was administered by oral gavage at concentrations of 0 (corn oil), 30, 100, or 300 mg/kg to 20 mated New Zealand White Rabbits/group on days 8 through 20 of gestation. Food consumption, body weight gain and feces were reduced in the high dose group. Maternal NOEL = 100 mg/kg. **No adverse effects**; Developmental NOEL = 300 mg/kg. ACCEPTABLE. (Kishiyama, Kellner and Gee, 11/3/93).

**020 045104, "A Teratology Study in New Zealand White Rabbits with Ro-Neet", (Wilczynski, V.M., Stauffer Environmental Health Center, Report T-12709, 4-29-86). Ro-neet Technical, 97.6%, was given by gavage to groups of 15 to 18 New Zealand White rabbits at dose levels of 0 (corn oil vehicle control), 10, 37.5 or 150 mg/kg/day on days 7-19 of gestation. At 150 mg/kg/day, there was a statistically significant decrease in maternal weight gain on days 7-10 of gestation, which was accompanied by a decrease in food consumption. At the same dose level, an increased number of fetuses was reported to have reduced bladder size. Maternal NOEL = 37.5 mg/kg/day (decreased maternal weight gain and food consumption); Developmental NOEL = 37.5 (reduced bladder size). Since the biological significance of reduced bladder size is questionable, and not an infrequent finding in control rabbits, the Developmental NOAEL is set greater than 150 mg/kg/day. The study is ACCEPTABLE and there is no indication of a possible adverse health effect (J. Parker, 9/6/86; G. Chernoff, 7/20/90).

GENE MUTATION

019 037081, "Mutagenicity Evaluation of Ro-neet Technical CGB-2201, Final Report", (Litton Bionetics, 10-77). Cycloate, lot no. CGB-2201 was assayed in Salmonella typhimurium, strains TA1535, TA1537, TA1538, TA98 and TA100 for revertants. The plate incorporation method was used at 6 concentrations from 0 to 5 ul/plate with and without activation. No increase in reversion rate. UNACCEPTABLE**.** Only 1 plate per concentration. (Gee, 1/22/86).

019 037082, "Mutagenicity Evaluation of Ro-neet Technical CGB-2201 Final Report", (Litton Bionetics 10-77). Cycloate, purity not stated, tested with Saccharomyces, strain D4, at 0 to 5 ul/plate. No adverse effect; UNACCEPTABLE. Single plate/concentration, test material not described. (Gee, 1/22/86).

** 019 037084, "Ro-neet Technical Mutagenicity Evaluation in Salmonella typhimurium", (Stauffer Environmental Health Center, 2-8-85). Cycloate, 97.6% was assayed in Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 with and without activation at 0, 0.012, 0.037, 0.111, 0.333 and 1.0 ul/plate. No increase in reversion rate. ACCEPTABLE**.** (Gee 1/23/86).

036 071076, "Mutagenicity Evaluation in L5178Y Mouse Lymphoma Multiple Endpoint Test Forward Mutation Assay", (ICI Americas Inc., 7/29/88, Study T-12045). Mouse Lymphoma L5178Y cells exposed for 4 hours in duplicate cultures to RO-NEET Technical (Lot No. WRC 4921-12-9, 97.6%) at dose levels between 0.0050 and 0.0600 ul/ml in a single assay without activation and at dose levels between 0.010 and 0.080 ul/ml in six repeat assays with activation; 48 hour or longer expression time. **Possible adverse effect-**dose-related increases in mutation frequency with activation; UNACCEPTABLE, Cannot be upgraded**-**the assay without S9 was not repeated. (Davis 11/8/88).

Summary**:** Two Ames assays and a yeast assay were all negative. Of these, one Ames assay was acceptable. The mouse lymphoma assay was unacceptable only because there was no confirmatory repeat assay in the absence of activation. Since there were repeat assays with activation, the positive findings have validity. Therefore, a possible adverse effect is identified. This is consistent with the positive findings in the other two mutagenicity categories. Davis, 11/9/88.

CHROMOSOME MUTATION

** 041 088831, "Cycloate: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes", (J. M. Mackay, ICI Central Toxicology Laboratory, Report No. CTL/P/3107, Study No. SV0383, 8/9/90). Cycloate technical, purity 98.1%, was assayed at concentrations of 10, 50, or 100 ug/ml in the presence and absence of metabolic activation (S-9 Mix) for clastogenic potential in human lymphocytes in whole blood. Blood was from 1 male and 1 female donor. Dimethylsulfoxide (solvent), mitomycin C and cyclophosphamide (positive) treatments served as controls. Treatment time was for 3 hours after cells were incubated for 44 hours from initiation. Reduced mitotic index was indicated for the mid and high dose groups. No adverse effect indicated. Cycloate treatments did not induce chromosomal aberrations in human lymphocytes. ACCEPTABLE. (Kishiyama and Gee, 1/4/91)

** 019 037085, "Ro-neet Technical Mutagenicity Evaluation in Bone Marrow Micronucleus", (Stauffer Environmental Health Center, 8/6/85). Ro-neet Technical, lot no. WRC 4921-12-9, 97.6%, was given by gavage to CD1 mice, 5/sex/group, at 0 (corn oil), 1000, 1500 or 2000 mg/kg. Sacrifices were at 24, 48 and 72 hours. No increase in micronuclei reported. Initially

reviewed as unacceptable based on the lack of individual data. These have been submitted as Record No. 54071, upgrading the study to ACCEPTABLE. (Gee 1/23/86, 7/10/87).

028 054071, Supplemental to 019 037085. Duplicate of 037085 plus appendices including individual data. (Gee 7/10/87).

**** 019 037083**, "Ro-neet Technical Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Cytogenetic Assay", (Stauffer Environmental Health Center, 8-6-85). Cycloate technical, 97.6%, lot no. WRC 4921-12-9. Mouse lymphoma cells were exposed to 0 to 0.10 ul/ml for 4 hours, with and without rat liver activation; 2 trials, 50 cells/conc. scored per culture; **Possible adverse effect -** increased chromosomal aberrations (structural and numerical) with activation. ACCEPTABLE. (Gee 1/22/86).

SUMMARY: A recent publication by E. D. Thompson in Environmental Mutagenesis 8: 753 (1986), compared the results in vitro for cytogenetics with in vivo results for cytogenetics or micronucleus formation for 216 chemicals. He concluded that 97% of the in vivo clastogens are positive in vitro but that the in vitro tests have a high incidence of "false positives" and a positive effect should be confirmed in animal studies. The author, however, did not use the stringent criteria used by EPA's Gene-Tox reports so the adequacy of each of the studies was not addressed. In the case of cycloate, an acceptable in vivo test is on file. There is no assurance, however, that the bone marrow is a target tissue while there is evidence for neuromyopathy. Since a positive effect was noted in both the chromosome aberration test and the sister chromatid exchange assay (see below), albeit in the same cell line, there is a suggestion that cycloate may be a clastogen or DNA damaging agent. (Gee 7/13/87).

DNA DAMAGE

**** 019 043229,** "Ro-neet Technical Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Cytogenetic Assay", (Stauffer Environmental Health Center, 8-6-87). Cycloate technical, 97.6%, lot WRC 4921-12-9, was tested for sister chromatid exchange in mouse lymphoma cells. Cells were exposed to 0, 0.005 to 0.06 ul/ml for 4 hours with and without rat liver activation. Forty cells/conc were evaluated. **Possible adverse effect-**SCE's per cell increased in the presence of S9 at 0.005, 0.01, 0.04 and 0.06 ul/ml. ACCEPTABLE. (Gee 1/22/86).

NEUROTOXICITY

** 008 024954 and 010 037061, "Acute Delayed Neurotoxicity Study with Technical Ro-neet in Adult Hens", (Stauffer Toxicology Lab, 7-10-79). Cycloate, 98.8%, lot no. CGB-2201 and WRC 4921-12-5, was administered to groups of 12 hens at 0, 10,170 mg/kg, single dose by oral gavage or 3,051 mg/kg administered on each of 5 days; dose repeated after 21 days. No adverse effect - No neurotoxicity reported. ACCEPTABLE. (Gee, 8/23/85, 1/21/86).

** **212-067, -088 126216, 154644** Rattray, N. "Cycloate: Acute Neurotoxicity Study in Rats" (Zeneca Central Toxicology Laboratory, Report No. CTL/P/3952, 7/15/93, first revision dated 12/6/95). Cycloate (97.6% purity, lot no. 4921-12-17) was administered by single oral gavage to 10 Alpk:APfSD rats/sex/dose at levels of 0, 200, 750 or 2000 mg/kg; rats were observed for 14 days. High-dose rats showed reduced food consumption during week 1, muscular rigidity in response to touch and decreased brain weight (females only). **Possible Adverse Effects:**

CNS lesions (necrosis of pyramidal neurons in the pyriform cortex and of granular cells in the dentate gyrus). In the initial report, the NOEL for females was 200 mg/kg, based on a single low-dose male showing necrosis of the pyriform cortex. In the revised report, the pathology re-evaluation found necrosis in 1 female and no male. Hence, the basis of the lack of a clear NOEL was changed from male rats to females. Another incidence change was reported at 750 mg/kg with the incidence of cell necrosis increasing in males from 3/5 to 4/5. At 2000 mg/kg, the change was primarily to change the grade from"marked" to "moderate". The previous reviews evaluated study as unacceptable but possibly upgradeable with submission of positive control data (historical) and justification of dose levels. Kellner and Gee, 10/13/93. Documents 212-071 through -075 were submitted for historical control data and dose justification (see 1-liner below). Submission of the first revision of the report [-088, 154644], containing both summary tables for home cage/handling observations and FOB and a greatly expanded Appendix 2 of individual data for these areas upgrades the study to ACCEPTABLE status. (Gee, 10/1/98)

212 -071 through -075 Record #'s 127762, -763,-764,-765,-766,-859; addendum to 212-067 126216. Five volumes of historical positive control data and dose level justification were submitted in response to deficiencies in an acute neurotoxicity study in rats (-067:126216). Dose level justification was acceptable; histopathology methods and motor activity tests performed on positive controls were also acceptable and conformed to U.S. EPA neurotoxicity guidelines. In contrast, the functional observation battery (FOB) for these rats did not conform to guidelines and showed the same deficiencies as in study -067:126216. The acute neurotoxicity study for cycloate remains unacceptable. Kellner, 12/14/94. The study has been upgraded - see above 1-liner. Gee, 10/5/98.

212-090 154652 "First supplement to trimethyltin chloride: Neurotoxicity study in rats." (Allen, S. L., CTL/P/3658, Zeneca Central Toxicology Laboratory, 11/3/95) Supplement to 127765 in -074. The supplement consists of Tables 1A and 1B and Appendix 1 (individual data). The expanded Table contains negative as well as positive results for the FOB portion of the study. Table 1A consists of home cage/open field observations and 1B consists of manipulative/in the hand observations. The original report did not report the negative results. Days of reporting were -1, 8, 15, 22 and 29. No worksheet. Gee, 10/5/98.

212-092 "First Supplement to Acrylamide: Neurotoxicity Study in Rats." (M. D. Stonard, Zeneca Central Toxicology Laboratory, UK, CTL/P/2226, 10/31/95) Supplement to 212-075, #127766. The supplement consists of pages 335 - 738 containing all clinical observations, including negative results. The original report contained only positive findings for days -1, 8, 15, 22 and 29. The Table 1A contains home cage/open field assessments and 1B the manipulative/ in the hand assessments. The Appendix contains the individual results. No worksheet. Gee, 10/5/98.

**** 212-069, 089 126741, 154646** Horner, S. "Cycloate: Subchronic Neurotoxicity Study in Rats" (Zeneca Central Toxicology Laboratory, Report No. CTL/P/4053, 10/22/93, Revised 11/14/95). Cycloate (97.6% purity, lot no. 4921-12-17) was administered in the diets to 12 Alpk:APfSD rats/sex/dose at levels of 0, 40, 400 or 4000 ppm for 13 weeks. Mid- and high-dose rats showed reduced food consumption and weight gain; high-dose females had urinary incontinence, increased response to touch, and upward curvature of the spine. High-dose males showed lower landing foot splay values. **Possible Adverse Effects:** CNS lesions (neuronal cell necrosis in localized areas of the dentate gyrus) in mid and high-dose females. Mid- and high-dose females also showed reduced brain weight and reduced locomotor activity. NOEL = 40 ppm. Unacceptable. Possibly upgradeable with submission of positive control data (historical) and justification of dose levels. Kellner and Gee, 11/8/93. The revised report

contains more details for clinical observations, handling observations and pathology. In addition, volume -090 contains supplemental data for the positive control of trimethyltin chloride. The study is upgraded to ACCEPTABLE status based on the revised report and supplental data. Gee, 10/5/98.

035 067692, "Neurotoxicity Study with Ro-Neet in Rats", (ICI Americas Inc., 3/10/88). RO-NEET Technical (Lot # WRC No. 4921-12-9, 97.6% purity by weight), administered by oral gavage to groups of 20 female Sprague-Dawley rats; for functional and neuropathologic changes, 3 treatment groups (400 mg/kg/day for 3 days, 220 mg/kg/day for 9 days, or 120 mg/kg/day for 27 days) with 3 associated vehicle control groups; 10 rats of each group were necropsied 1 day after the last treatment & 10 were necropsied 28 days after the last treatment; for biochemical changes, 10 females per group were treated with the test material or corn oil with the same dose levels and time periods as above; positive controls were TOCP & carbaryl. No adverse effect: stained coats & reduced hindlimb grip were reversible; RBC cholinesterase reduced $\leq$ 22% & brain neurotoxic esterase reduced $\leq$ 14%. Supplemental Study. (Davis 9/27/88).

037 074206, This document contains individual histopathology data, a summary table of Wallerian degeneration of sciatic nerves for rats, and addenda to protocols for assays for serum, brain, and erythrocyte cholinesterase and neuropathy target esterase activities for doc. # 212-035, rec. # 067692. Examination of these data did not result in any changes of study status (Morris, 2/21/90).

212 087 154643 “Thiocarbamates: Selective re-examination of neuropathology” (Chalmers, D. T., S. J. Duffell and S. A. Horner, CTL/P/4618, Zeneca Central Toxicology Laboratory, UK, 3/28/95) This is a report of the re-examination of slides of the pyriform cortex and dentate gyrus of the brains of rats given acute or subchronic doses of 6 thiocarbamates: cycloate [CTL/P/3952, CTL/P/4053, CTL/P/4432], EPTC, molinate, pebulate, vernolate and butylate, for neuronal cell necrosis to confirm the earlier pathological evaluations. Two pathologists reviewed the slides and discussed any disagreements. Results with cycloate confirmed treatment-related effects following acute exposure at 200, 700 and 2000 mg/kg. Following subchronic exposure, effects were noted at 400 and 4000 ppm cycloate. The data on the other thiocarbamates have been noted in this review in tabular form taken from the report. It should be noted that no evidence of cell necrosis was seen in the subchronic studies with molinate, pebulate, vernolate or butylate. Twelve photographs were included. Submitted as supplemental data following the development of criteria for determining neuronal cell necrosis. SUPPLEMENTAL. (Gee, 9/30/98)

OTHER

026 053454, "Subchronic Inhalation Study With RO-NEET Technical in Rats", (Knapp, H.F. and R. W. Thomassen, Stauffer Chemical Co., Report T-12621, 11/6/86). RO-NEET Technical, 97.6%, Lot #EHC-0469-09, was administered to groups of 18 male and 18 female Sprague-Dawley CD rats by inhalation at dose levels of 0, 1.2, 12, or 120 mg/m^3 for 6 hours per day, 5 days a week, for a total of 68 to 71 days. There was a significant increase in the incidence of nasal respiratory epithelial hypertrophy/hyperplasia at all dose levels tested. At the high dose, food consumption, body weight, and absolute brain weights were decreased, while the incidence of nervous system alterations, salivation, tremors, chromodacryorrhea, and chromorhinorrhea were increased. The NOEL < 1.2 mg/m^3 (altered nasal respiratory epithelium). The NOEL for neural damage is 12 mg/m^3 (G. Patterson, 3/17/87, G. Chernoff,

7/24/90 and Gee, 3/21/91).

** 027 053455, "Subchronic Inhalation Study with RO-NEET Technical in Rats", (Knapp, H.F. and R.W. Thomassen, Stauffer Chemical Co., Report T-11705, 11/7/84). RO-Neet Technical, 96.8%, Lot #EHC 0469-09, was administered to groups of 18 male and 18 female Sprague-Dawley CD rats by inhalation at dose levels of 0, 2.5, 17, or 120 mg/m^3 for 6 hours per day, 5 days per week, for a total of 65 days. There was a significant increase in the incidence of nasal respiratory epithelial hypertrophy/hyperplasia at all dose levels tested. At the mid and high dose levels, there was a significant decrease in food consumption, body weight, and absolute brain weight in both males and females. The incidence of chromorhinorrhea, chromodacryorrhea, and rough hair was significantly elevated at the high dose tested, and the absolute ovarian weight was decreased. The grade of gynecomastia (hypertrophy/hyperplasia of the male mammary gland) was significantly increased in high dose males, but could not be evaluated at the low and mid-dose because of a loss of specimens in a laboratory fire. Initial review (GTP) considered the NOEL to be 2.5 mg/m^3. Upon rereview, the NOEL was lowered to: NOEL < 2.5 mg/m^3 (alterations in the nasal epithelium). (G. Patterson, 3/18/87 and G. Chernoff, 7/23/90).

SUMMARY: Two subchronic rat inhalation studies were submitted for CDFA review. In the first (CDFA Record No. 053455), the major findings were gynecomastia and hypertrophy/ hyperplasia of the nasal respiratory epithelium. The results for gynecomastia were confounded by the lack of low and mid dose samples, presumably because of a laboratory fire. In the second study, gynecomastia was not observed in any of the control or treated males. The major finding from this study was hypertrophy/hyperplasia of the nasal respiratory epithelium. This study was thought by the authors to be compromised by a concurrent disease in all the animals which caused alterations in the nasal epithelium. The presence of the disease was never verified. Based on these findings, a definitive NOEL for hypertrophy/hyperplasia of the nasal respiratory epithelium cannot be established (G. Chernoff, 7/24/90).

048 092416, 092417 Response of ICI to questions raised by Medical Toxicology on the above two subchronic inhalation studies. No data. No worksheet. Gee, 5/2/91.

056 113175 "Cycloate: 21 Day Sub-Acute Inhalation Toxicity Study in the Rat, (Lewis, R.W. and R.J. Parr-Dobrzanski, ICI Americas Inc., Report No. CTL/P/3646, 2/10/92); Cycloate Technical, 97.8%; inhalation exposure, nose-only-6 hours/day, 5 days/week for 3 weeks; Groups I (main study) and II (30 day recovery), 5 animals/sex/group/exposure concentration; Exposure concentrations: control, 2.0, 20.0, and 200 mg/m^3; Observations: (treatment-related) salivation evident at sometime during exposure period (20.0 mg/m^3-5/20, 200 mg/m^3-20/20), not observed during recovery period, **possible adverse effect:** reduced splay response-(0-1/19, 2.0 mg/m^3-3/19, 20.0 mg/m^3-2/19, 200 mg/m^3-5/19) (ie. numbers do not include animals suffering from cerebellar hypoplasia), reduced response evident in some animals from the initiation of the study to the end of the recovery period; other signs noted in the treated animals were common to the control group as well (chromodacryorrhea, pilorection); Necropsy: treatment-related mottling of lungs, not evident in recovery group; Micropathology: no treatment-related lesions in either the main study or the recovery group; NOEL = 20.0 mg/m^3 (reduced splay response at 200 mg/m^3); Study unacceptable (nervous tissue preparation was not adequate to evaluate potential micropathology). (Moore, 4/3/92).

212-064 124137 Coombs, D. "Cycloate: 3-Week Inhalation Neurotoxicity Study in Rats" (Huntingdon Research Centre, England, Zeneca Report No. CTL/C/2934, 6/6/93). Cycloate (97.6% purity, lot no. 4921-12-17) was administered by inhalation (snout only) to 10

Sprague-Dawley rats/sex/dose at levels of 0, 2, 20 or 200 mg/m^3 6 hours/day, 5 days/week for 3 weeks. Reduced body weight gain in mid- and high-dose females and increased hind limb grip strength in all dosed female groups and mid- and high-dose males was noted. **Possible Adverse Effects:** Reduced brain weight in all female groups (significant only at high-dose) and increased incidence of axonal degeneration in the sciatic nerve (sciatic notch and mid-thigh) and tibial nerve in high-dose males; axonal degeneration in the trapezoid body of the brain of a high-dose male was also seen. NOEL < 2 mg/m^3 (reduced female brain weights at all dose levels). UNACCEPTABLE. No dose-level justification, positive control data or histopathological examinations of neural tissue from low and mid-dose groups; inappropriate analysis and reporting of brain-weight data. Possibly upgradeable with submission of missing range-finding, historical positive control and histopathology data; also, justification for adjustments made on brain weight data is needed. Kellner and Gee, 10/13/93.

212-061 118228 Protocol for Record No. 124137, above.
212-062 118546 Protocol amendments for Record No. 124137, above.

Summary: Evidence of adverse compound-related effects in the nervous system of rats was demonstrated. In an acute neurotoxicity study (-067:126216), reduced female brain weight and neuronal cell necrosis in the dentate gyrus was seen. Another possible compound-related effect, namely axonal degeneration in the sciatic nerve, was dismissed by the author as an "incidental feature of the peripheral nervous system of a number of strains of rat, including those of the Alderley Park strain, and was not related to exposure to cycloate". Another study performed by this laboratory (-064: 124137; 3-week inhalation) also suggested that a slight effect on sciatic nerve may have been compound-related. According to the author "one high dose group rat (#35 male), killed at termination, showed moderate axonal degeneration in sciatic nerve (sciatic notch and mid-thigh) and tibial nerve, and also minimal axonal degeneration in the trapezoid body in the brain". In addition to axonal degeneration of the sciatic nerve and spinal cord, effects on hind limb splay and brain weight reductions in females were demonstrated.

These effects were confirmed in a subchronic feeding study (-069:126741). Both the male and female high-dose groups and mid-dose females showed neuronal cell necrosis in localized areas of the dentate gyrus; females showed significantly reduced brain weights (at 400 and 4000 ppm) and reduced motor activity (4000 ppm). Sciatic nerve fiber degeneration (although minimal) was reported in about half of the 4000 ppm rats whose tissues were examined microscopically. As was done in the 3-week inhalation study, the author adjusted the brain weights using the final body weight (analysis of covariance, ANCOVA). Despite this adjustment, lower female brain weights were still statistically significant at the 400 and 4000 ppm dose levels. Absolute (but not the adjusted) mean brain weight for males at 4000 ppm was also significantly lower. Kellner, 11/8/93.

212-078 133350 Parr-Dobrzanski, R.J., "Cycloate: 21 day sub-acute inhalation toxicity study in the rat", Zeneca Central Toxicology Laboratory, Cheshire, CTL/P/4432, 8/24/94. Crl:CD(SD)BR rats, 18/sex/exposure level, were exposed to cycloate for 6 hr/day, 5 days/week for 3 weeks at concentrations of 0, 1.2, 12, or 120 ug/l. In addition, a recovery study was performed, in which the same group sizes were exposed to the same concentrations, but the latter groups were taken off treatment for 70 days before sacrifice. Rats were evaluated for clinical observations, body weight gain, food consumption, and for histopathological changes in suspected target tissues (respiratory tract, central and peripheral nervous system). No NOEL was observed in this study, however dose-response relationships were typically well-defined. Treatment effects, especially in the upper respiratory tract, were more pronounced in this study than in earlier inhalation studies (such as Record Nos. 113175 and 124137, both of which were

"nose only" exposures). Several changes in the nasal cavity (rhinitis, goblet cell hyperplasia, respiratory epithelial cell hyperplasia, and transitional cell hyperplasia), and in the larynx (squamous epithelial cell hyperplasia) were observed in all dose levels in females, and in most cases also in males. Study is valid for its intended purposes as a follow-up study (not applicable to guidelines for a particular study type). Study shows "possible adverse effects", based primarily upon the histopathological changes at low dose levels in the upper respiratory tract, and secondarily upon neuronal cell necrosis in the pyriform cortex of the cerebrum in both sexes at 120 mg/l, and an equivocal response in females at 12 mg/l. Also, slight brain weight reductions at 120 mg/l were observed, consistent with several dietary and inhalation rat studies. Aldous, 12/27/94.

** 053 093251 “Cycloate: 21-day dermal toxicity to the rat.” (D. L. Kinsey and A. M. Leah, ICI Central Toxicology Laboratory, Cheshire, UK, Study # LR0551, 9/18/91); 822; cycloate (Y06375/001, 97.8% purity); 6 hour exposure/day at 5 days/week amounting to 21 dermal applications over a 30-day period; 0 (sham control), 10, 50 or 200 mg/kg; 5 rats/sex/dose; 2-high-dosed rats (1F/1M) were killed in extremis; both deaths were considered to be due to stress caused by bandaging and exacerbated by skin irritation; clinical signs: dose-related incidence and severity of skin irritation in mid- and high-dosed rats consisting of desquamation, erythema, edema and thickening of the skin; **no systemic adverse effects**; high-dosed males exhibited a slight decrease in mean body weight ($p<0.05$, 94% of control) with minor changes in food consumption; no treatment-related effects in clinical chemistry, hematology, organ weights, macroscopic and microscopic findings; NOEL (M/F) = 10 mg/kg (based on incidence and severity of skin irritation); **acceptable;** (Leung, 11/6/91).

212-091 154653 “First supplement to cycloate: 21 day dermal toxicity to the rat.” (Lees, D., Zeneca Central Toxicology Laboratory, CTL/P/3352, 7/8/94) Supplement to acceptable study in -053 093251 above. The supplement consists of histopathological examination of the sciatic nerve and transverse sections of the cervical, lumbar and thoracic spinal cord from the control and high dose (200 mg/kg) rats using light microscopy, 5/sex/dose. There were no treatment-related findings. No worksheet. Gee, 10/5/98.